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Emerging Therapeutic Implications of *STK11* Mutation: Case Series

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Abstract

STK11 was first recognized as a tumor suppressor gene in the late 1990s based on linkage analysis of patients with Peutz-Jeghers syndrome. *STK11* encodes LKB1, an intracellular serine-threonine kinase involved in cellular metabolism, cell polarization, regulation of apoptosis, and DNA damage response. Recurrent somatic loss-of-function mutations occur in multiple cancer types, most notably in 13% of lung adenocarcinomas. Recent reports indicate that *KRAS*-mutant non-small cell lung cancers harboring co-mutations in *STK11* do not respond to PD-1 axis inhibitors. We present three patients with *STK11*-mutated tumors and discuss the proposed mechanisms by which germline and somatic alterations in *STK11* promote carcinogenesis, potential approaches for therapeutic targeting, and the new data on resistance to immune checkpoint inhibitors. ***The Oncologist*** 2020;25:733–737

Key Points

- *STK11* is a tumor suppressor gene, and loss-of-function mutations are oncogenic, due at least in part to loss of AMPK regulation of mTOR and HIF-1-α. Clinical trials are under way, offering hope to patients whose *STK11*-mutated tumors are refractory and/or have progressed on chemotherapeutic regimens. Whether gastrointestinal cancers with *STK11* loss of function will show the same outcome and potential refractoriness to immune therapy that were reported for lung cancer is unknown. However, physicians managing such patients should consider the experience in lung cancer, particularly outside the context of a clinical trial.
- In the CheckMate-057 trial lung tumors harboring co-mutations in *KRAS* and *STK11* had an inferior response to PD-1 axis inhibitors. Coupled with the observation that *STK11*-mutated tumors were found to have a cold immune microenvironment regardless of *KRAS* status, the conclusion could extend to *KRAS* wild-type tumors with *STK11* mutation. Current data suggest that the use of PD-1 axis inhibitors may be ill advised in the presence of *STK11* mutation.

Patient Story No. 1

A 73-year-old woman presented with abdominal pain. Computed tomography (CT) scan was suspicious for a 6-cm intrahepatic malignancy with peripheral enhancement and multiple bilobar hypodense satellite lesions. A biopsy confirmed cholangiocarcinoma. She completed three cycles of chemotherapy with gemcitabine and cisplatin; the carbohydrate antigen 19-9 increased from 3,500 to 133,000 U/mL, and restaging scans demonstrated enlarging liver metastases. A targeted next-generation sequencing (NGS) panel of 467 cancer-associated genes revealed an inactivating frameshift variant in *STK11* (NM_000455.4: c.842dup, p.L282fs) with 49% allele fraction. No variants were identified in *KRAS*, *TP53*, *IDH1*, *IDH2*, or *FGFR2*. Tumor mutational burden was 3.15 mutations per megabase. The patient died 5 months after diagnosis, after three cycles of chemotherapy and radiation to the primary.

Patient Story No. 2

A 78-year-old man with history of human immunodeficiency virus, extensive tobacco use of 60 pack-years, and multiple comorbidities presented with gross hematuria, anorexia, and

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weight loss. He underwent cystoscopy and transurethral resection of a bladder tumor; histopathology was atypical for urothelial carcinoma. He was found to have multiple lung lesions on positron emission tomography–CT; he refused biopsy, and pembrolizumab was initiated. Restaging scans demonstrated significant progression of disease in the lungs. Biopsy was then performed, with pathology consistent with lung adenocarcinoma. Sequencing demonstrated a concordant *KRAS* mutation in lung and bladder tumor as well as an inactivating nonsense mutation in *STK11* (p.E120*). The post-pembrolizumab Tumor Proportion Score (TPS) was <1%, and no variants in *TP53* or *KEAP1* were identified.

After pembrolizumab monotherapy, the patient underwent stereotactic radiation to several of the dominant lung lesions and three cycles of pemetrexed and pembrolizumab, but he developed further disease progression and died.

Patient Story No. 3

An 82-year-old man presented with jaundice, abdominal pain, and significant weight loss. CT scan demonstrated intra- and extrahepatic biliary ductal dilatation. Endoscopic retrograde cholangio pancreatography and biopsy were performed. The pathology was consistent with ampullary cancer, and an NGS panel demonstrated an inactivating mutation in *STK11* (NM_000455: c.374+1A>G). He subsequently underwent a Whipple procedure. Liver metastases were noted 4 months after surgery during adjuvant chemotherapy.

Functional and Clinical Significance of *STK11* Mutations

The serine-threonine kinase 11 (*STK11*) gene, first identified by Chugai Pharmaceuticals in 1996, is located on the short arm of chromosome 19 and consists of nine coding exons and one noncoding exon. STK11 protein, also known as LKB1, is composed of 433 amino acids and is widely expressed in all tissues. LKB1 has a pivotal role in cellular energy metabolism. Under low ATP conditions, LKB1 forms a heterotrimeric complex with the pseudokinase STE20-related adaptor alpha and a scaffolding protein MO25α, resulting in allosteric activation of its kinase domain. LKB1 is then able to phosphorylate and activate 5' AMP-activated protein kinase (AMPK) [1]. AMPK activation in turn phosphorylates and inactivates enzymes involved in the synthesis of macromolecules while promoting catabolism to maintain cellular energy balance. Among the critical targets repressed is mTOR complex 1 (mTORC1), which occupies a central role in controlling cell growth. Loss of LKB1/AMPK signaling results in aberrant activation of mTOR and thereby promotes tumorigenesis [2, 3].

Loss of LKB1 activity results in reduced use of macromolecule energy stores despite low ATP conditions, with reprogramming of cellular metabolism to increase glucose and glutamine use via the HIF-1-α pathway. Normally induced under low oxygen conditions, the transcription factor HIF-1-α can be constitutively expressed in human cancers even in the absence of hypoxia [4]. In a growing tumor, increased HIF-1-α shifts glucose metabolism from oxidative phosphorylation to the glycolytic pathway to maintain energy supply, and tumor cell proliferation continues despite a hypoxic environment [5]. LKB1 inactivation has also been associated with genomic instability and loss of repression of cancer invasiveness through a yet to be fully characterized mechanism [6, 7].

Germline *STK11* mutations are causal for Peutz-Jeghers syndrome, an autosomal dominant disorder resulting in mucocutaneous hyperpigmentation, hamartomas throughout the gastrointestinal tract, and a predisposition for breast, lung, pancreas, and gastrointestinal malignancies including cancers of the colon and small bowel. Like *TP53*, loss of heterozygosity (LOH) is typically observed in emerging cancers in patients with germline alterations. The incidence of pancreatic cancer is increased ∼130-fold [8].

Analyzing The Cancer Genome Atlas (TCGA)'s PanCancer data set via cBioPortal.org, point mutations or small indels are found in 1.5% of the 10,967 (predominantly early stage) tumors, including 13% of lung adenocarcinomas, 2.8% of cholangiocarcinomas, and 2.2% of pancreatic adenocarcinomas, frequently co-occurring with copy number alterations or deletions that involve *STK11* [10–12]. *STK11* point mutations occur across the gene span and are frequently nonsense or frame shift mutations, and most are predicted to be oncogenic (Fig. 1) [13]. Other data sets have noted alteration rates in advanced adenocarcinoma of the lung as high as 30% [14, 15]. LOH at chromosome 19p was reported in 62% and homozygous deletion in 28% of 124 non-small cell lung cancer (NSCLC) samples [9]. Copy number alterations have also been observed in additional tumor types, including deep deletion in 3.25% of ovarian carcinomas.

Potential Strategies to Target the Pathway and Implications for Clinical Practice

Several approaches have been proposed to target *STK11*-deficient tumors based on preclinical observations and are summarized in Figure 2. By inhibiting complex I of the electron transport chain, metformin can induce metabolic stress and ultimately tumor cell death. Phenformin, a biguanide-like metformin, has been shown to reduce tumor size and induce prolonged survival of mice with tumors harboring *STK11* mutations [16]. Inhibitors of downstream signaling have also been proposed. Phenformin in combination with sapanisertib, a potent and selective mTOR inhibitor, was active in human cell lines harboring *KRAS*/*STK11* mutations as well as in mouse models of NSCLC [16]. In support of this, a single case study reported a near-complete response to everolimus in a patient with heavily pretreated metastatic breast cancer. Genomic profiling of a liver metastasis revealed a point mutation in *STK11* accompanied by LOH at 19p [17].

Drugs that target protein glycosylation and folding, thus inducing endoplasmic reticulum stress, such as tunicamycin and brefeldin A, and drugs that target glycolysis and induce metabolic stress such as 2-deoxyglucose (2-DG), may induce synthetic lethality in cancers with deficient LKB1/AMPK activity. A study in a murine model demonstrated that treatment with 2-DG decreased the growth of NSCLC tumors, with a statistically greater response to this drug in the *KRAS*/*STK11* co-mutated tumors (p = .0032) compared with wild-type tumors [18].

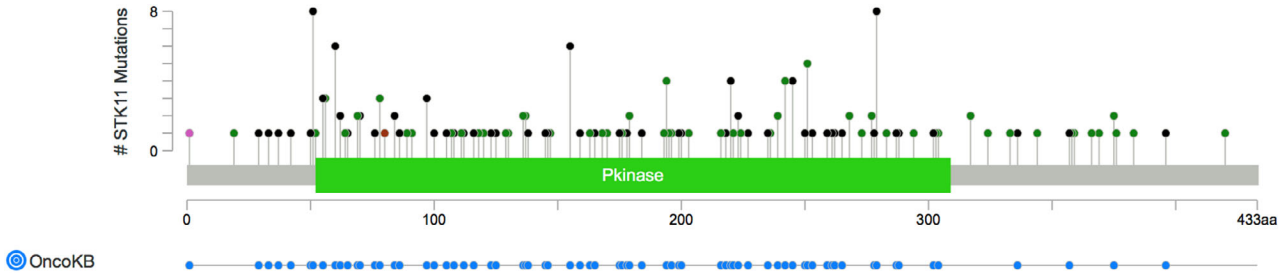

Figure 1. *STK11* mutation distribution. *STK11* mutations were found in 1.5% of samples in the TCGA PanCancer dataset available on cBioPortal.org [11, 12]. The mutations considered oncogenic are noted in blue circles.

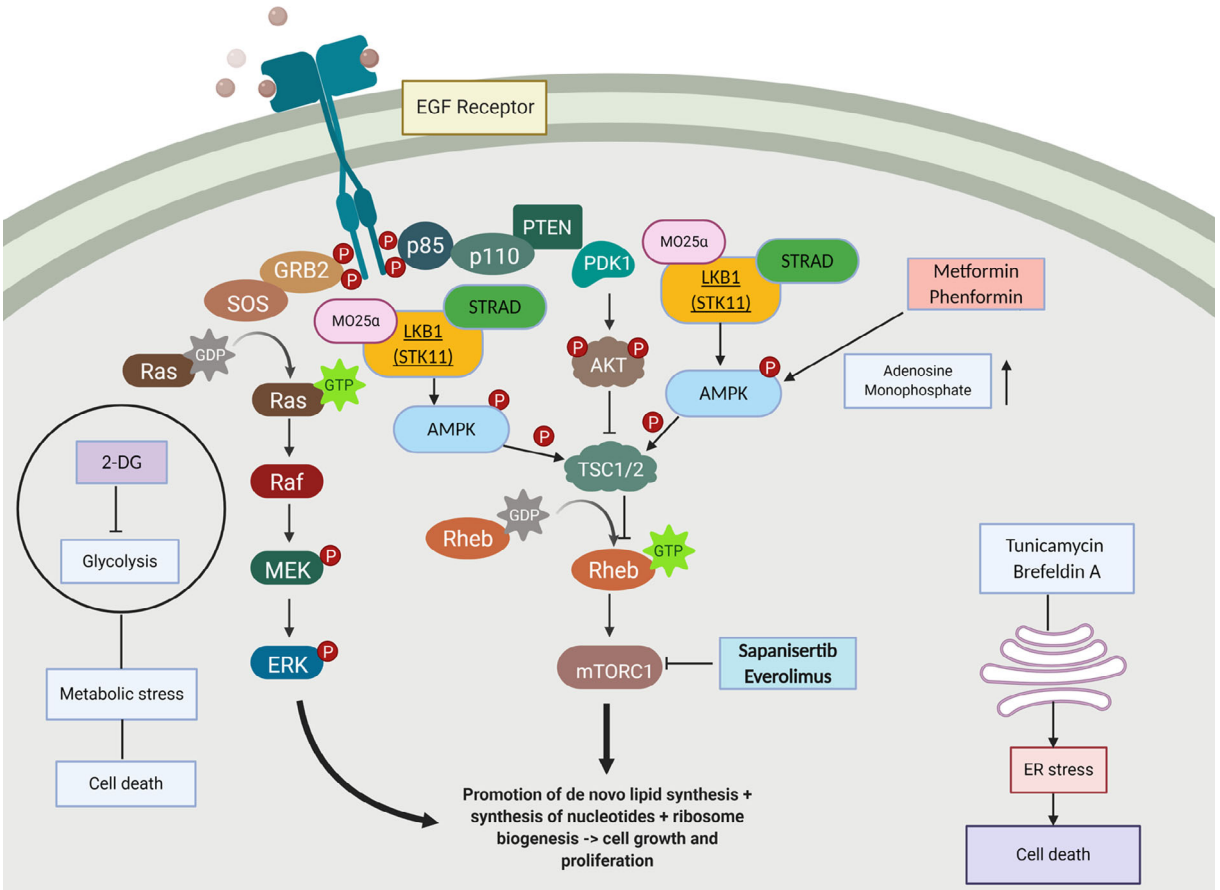

Figure 2. Targeting the pathways involved in *STK11/LKB1*. *STK11/LKB1* phosphorylates AMPK, leading to phosphorylation of TSC1/2, which then reduces Rheb activity thereby limiting activation of mTORC1. MTORC1 is an effector protein complex for cell growth and survival pathways. It is activated when Rheb is GTP-bound. Loss of *STK11* therefore reduces AMPK and TSC1/2 phosphorylation, releasing inhibition of Rheb and allowing activation of mTORC1. Several strategies to limit mTORC1 activation have been proposed. Metformin inhibits complex 1 in the mitochondria, which raises AMP concentration and ultimately inhibits activation of mTORC1. Tunicamycin and Brefeldin A induce stress on the endoplasmic reticulum and promote cell death. 2-DG leads to inhibition of glycolysis, causes metabolic stress, and ultimately cell death. Sapanisertib and everolimus inhibit mTORC1, thus inhibiting cancer cell survival.

Preclinical data also suggest that *STK11* alterations may confer resistance to standard therapy. Genetically engineered mice with *KRAS*-, *KRAS*/*TP53*-, or *KRAS*/*STK11*-mutant adenocarcinomas of the lung were randomized to receive docetaxel by intraperitoneal injection every other day, the oral MEK inhibitor selumetinib daily, or docetaxel in combination with selumetinib [19]. Mice with *KRAS* and concomitant loss of either *TP53* or *STK11* had markedly lower response rates to docetaxel (5% and 0%, respectively) than did those with *KRAS* alone (30%). Furthermore, combining docetaxel with selumetinib substantially improved response rates in the *KRAS* and *KRAS*/*TP53* cohorts but had more modest benefit in the *KRAS*/*STK11* models.

There is also suggestion of reduced survival in patients whose tumors carry *STK11* mutations. Skoulidis et al. reported three clusters of *KRAS*-mutant lung adenocarcinomas—bearing

Table 1. Overall survival in cancer subtypes with at least one *STK11* mutation using PanCancer database

	Cases with mutation(s) in *STK11*			Cases without mutation(s) in *STK11*			
Cancer type	Total cases, *n*	Deceased cases, *n*	Median survival, months	Total cases, *n*	Deceased cases, *n*	Median survival, months	*p* value
Lung adenocarcinoma	76	33	31.20	429	149	50.2	.0441
Lung SCC	5	4	10.8	473	200	55.7	.0009
Adrenocortical carcinoma	2	1	18.1	89	32	NA	.402
Cholangiocarcinoma	1	1	8.9	35	17	40.1	.0583
Bladder urothelial carcinoma	2	1	7.87	407	179	33.1	.942
Colorectal cancer	6	2	NA	524	108	83.24	.315
Invasive breast cancer	7	1	70	1,057	149	130	.812
Cervical SCC	13	6	18.7	278	61	101.8	.0686
Esophageal adenocarcinoma	4	1	7.8	178	75	26.3	.511
Stomach adenocarcinoma	6	2	4.5	425	165	30.9	.339
Head and neck SCC	7	3	NA	507	215	56.5	.714
Renal papillary cell carcinoma	2	0	NA	273	40	NA	.626
Hepatocellular carcinoma	1	1	8.6	364	126	59	.0144
Ovarian serous cystadenocarcinoma	1	1	20	518	304	45.1	.0723
Pancreatic adenocarcinoma	5	2	19.8	174	95	20.2	.816
Prostate adenocarcinoma	1	0	NA	493	10	NA	.900
Cutaneous melanoma	10	8	79	413	201	80.7	.956
Papillary thyroid carcinoma	1	0	NA	488	15	NA	.772
Uterine corpus endometrial carcinoma	15	2	107	501	83	NA	.621

Abbreviations: NA, not available; SCC, squamous cell carcinoma.

STK11 mutation (31%–34%), *TP53* mutation (44%–51%), or *CDKN2A/B* inactivation (17%–22%) [20]. Patients with *KRAS/STK11* co-mutation or *KRAS/CDKN2A/B* inactivation had poorer overall survival (OS) than patients with *KRAS/TP53* co-mutation [20]. Analysis of survival of patients with early stage lung adenocarcinoma in TCGA PanCancer data set also shows poorer OS in patients with *STK11* mutations. Furthermore, there may be a trend toward decreased median OS in several cancer types in TCGA (Table 1), with the caveat that the number of variants in most cancer types is small and not easily amenable to multiple regression analyses to determine if the effect of *STK11* is independent of cancer type, stage, and a multitude of other important clinicopathological covariates [11, 12].

To determine whether *STK11* affected prognosis after immunotherapy, investigators analyzed data from the landmark CheckMate-057 randomized phase III trial of nivolumab versus docetaxel in the second-line setting for advanced nonsquamous NSCLC [21]. Among 924 patients with lung adenocarcinoma in this trial, patients with concomitant somatic mutations in *KRAS* and *STK11* demonstrated a slightly shorter progression-free survival (PFS; 1.8 months vs. 2.7 months; $p < .001$) but a significantly shorter OS (6.4 months vs. 16 months; $p = .0015$) compared with patients with *KRAS*-only mutant tumors treated with immunotherapy. Interestingly, they noted that *STK11* alterations were associated with lack of expression of PD-L1.

To validate these findings, investigators queried genomic drivers of absent PD-L1 expression in lung adenocarcinoma using an independent Foundation Medicine data set. The results revealed alterations in *STK11* as the only gene significantly enriched in the PD-L1 negative group of lung adenocarcinomas ($p < .001$), indicating that *STK11* mutation is associated with PD-L1 absence irrespective of *KRAS* status. Skoulidis et al. also retrospectively identified 66 patients with nonsquamous NSCLC who were treated with PD-1 or PD-L1 inhibitors (pembrolizumab, nivolumab, atezolizumab, durvalumab, or tremelimumab) and reported that *STK11* mutations were associated with significantly lower overall response rate ($p = .026$) as well as significantly shorter median PFS (1.7 vs. 19.3 months, $p = .00012$) and median OS (11.1 vs. 26.5 months, $p < .0001$) [22]. As proof of concept, the authors demonstrated that bi-allelic disruption of *STK11* with clustered regularly interspaced short palindromic repeats directly induced resistance to immunotherapy in two *KRAS*-mutant murine models [21]. As to what might drive a poorer response to immunotherapy beyond the PD-L1 downregulation, investigators have described depletion of infiltrating cytotoxic T cells in both murine and human *STK11*-mutant tumors [21]; accumulation of neutrophils and cytokines, suppressing T-cell activity in the tumor microenvironment [23]; and competition of tumor cells with T cells for glucose, leading to T-cell hyporesponsiveness [24].

Trials targeting *STK11* are ongoing. There is an ongoing phase II trial recruiting patients with urothelial carcinoma whose advanced tumors harbor certain mutations associated with defects in DNA damage repair, including *STK11*. The patients receive olaparib after failure of a platinum-

containing chemotherapy regimen [25] Another clinical trial is under way to offer the glutaminase inhibitor CB-839 hydrochloride for treatment of metastatic or unresectable solid tumors harboring mutations in several genes involved in metabolism, oxidative stress, and mTOR regulation, one of which is *STK11* [26]. The LUNG-MAP study has recently opened an arm to treat patients with tumors bearing *STK11* mutations with talazoparib plus avelumab (NCT04173507).

Glossary of Genomic Terms and Nomenclature

Allele Fraction: In next-generation sequencing, the percentage of reads consistent with a specific [variant] allele.

Frameshift variant: An insertion or deletion of a number of base pairs (not a multiple of 3), which disrupts the triplet reading frame of the DNA sequence. This frequently leads to an inactive or prematurely truncated protein product.

Germline alteration: Detectable variation within germ cells that can be inherited by offspring.

LOH: Loss of heterozygosity; the complete deletion of one allele of a gene. LOH combined with one variant allele from a germline or somatic alteration is a common mechanism for loss of function of a tumor suppressor.

Non-coding exon: Regions of genes that are represented in mature mRNA but are ultimately not translated into protein.

Nonsense mutation: A mutation in which substitution of a single base pair results in a stop codon rather than a codon specifying an amino acid.

Somatic alteration: An alteration in the DNA that happens after conception in any of the cells of the body except the germ cells and is not inheritable.

Tumor mutational burden: A measure of the quantity of mutations identified in a particular tumor sample, usually expressed as alterations per megabase.

Tumor Proportion Score (TPS): Percentage of viable tumor cells showing partial or complete membrane staining at any intensity.

Tumor suppressor gene: A gene that makes a tumor suppressor protein, whose canonical function is to control cell growth and regulate cell division. Loss of function mutations in such a gene can contribute to carcinogenesis.

Author Contributions

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Provision of study material or patients: Bahar Laderian, Prabhjot Mundi
Collection and/or assembly of data: Bahar Laderian, Prabhjot Mundi
Data analysis and interpretation: Bahar Laderian, Susan Bates
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Final approval of manuscript: Bahar Laderian, Prabhjot Mundi, Tito Fojo, Susan Bates

Disclosures

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